REMARKS

Claims 35-39 are pending in the present application. In an Advisory Action mailed on February 23, 2006 ("Advisory Action"), Examiner maintains the rejection of Claims 35-39 from the Office Action of October 20, 2005 ("Office Action") under 35 U.S.C. § 112, first paragraph as allegedly not being enabled. Applicants respectfully disagree.

The Claimed Invention Meets the Requirements of 35 U.S.C. §112, first paragraph.

Claims 35-39 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly not being enabled. In particular, the Examiner asserts that the specification fails to provide any predictable use for the claimed compositions. (Office Action and Advisory Action). Applicant respectfully disagrees.

The Examiner acknowledges that the specification provides sufficient guidance with respect to the production of the multivalent compositions that are the subject matter of these claims. Office Action page 3). Thus, the Examiner's sole basis for alleging lack of enablement is the allegation that the specification has not provided any predictable use for the claimed method (Office Action, page 3). The Examiner has further asserted that the Applicant has not provided any arguments by of affidavit, declaration, or citation to references that show that one of skill in the art would be capable of practicing the invention without undue experimentation. (Advisory Action). Applicant respectfully disagrees with each of the Examiner's assertion.

The specification points to literature describing the successful use of tumor-specific immunoglobulin ("Ig") to immunize patients in order to invoke an immune response against the tumor cell (see, *e.g.*, page 82, line 28, citing Kwak *et al.* (1992) N. Engl. J. Med. 327:1209 ["Kwak"], *inter alia*). Applicant notes that the Kwak reference is incorporated by reference into the specification of the instant application (specification at page 104, lines 25-26). For the Examiner's convenience, a copy of Kwak is provided herewith.

As described in Kwak, B-cell tumors are composed of clonal proliferations of cells that synthesize a single type of antibody molecule that is expressed on the cell surface. (Kwak, page 1209, col 1). The idiotypic portion of this surface immunoglobulin serves as a specific marker for the tumor. (Kwak, page 1209, col 1). Kwak provides a complete description of methods for producing a purified B-cell lymphoma surface immunoglobulin, and for use of the purified Ig as

an antigen to invoke an immune response in an injected subject (see, *e.g.*, the METHODS section, p1209, col 2, to p1211, col. 1). The Ig produced by the method of Kwak (the "rescue fusion" method, described on p1210, col 1) represents a *single* Ig derived from the patient's tumor, *i.e.*, it is monovalent. (See, *e.g.*, specification at page 89, lines 5-11).

Kwak demonstrated that monovalent immunoglobulin derived from a patient's own tumor, when injected back into the patient from whom it was derived, produced a sustained idiotype-specific immunological response in that patient, in 7 of 9 patients tested. (Kwak, Abstract on page 1209). Thus, Kwak demonstrates that the use of tumor-cell derived immunoglobulin to invoke a specific immune response in a subject is not unpredictable. Further, Kwak observed complete tumor regression in the patients that had tumors at the time of their initial injection. (Kwak, page 1213, col 2 to 1214, col 1).

The present invention is directed toward producing a multivalent composition derived from a subject's tumor and representative of somatic variants found within the subject's tumor. Kwak acknowledged that B-cell tumors can comprise tumor cells with mutated forms of their surface immunoglobulin (Kwak, p 1214, col 1, and as further described by the Applicant in the instant specification at page 52, lines 18-26). However, as described above, the Ig produced by the method of Kwak is monovalent and does not represent the full complexity of Igs expressed in tumors that contain somatic variants. (Specification, page 89, lines 9-11). Applicant submits that one of skill in the art, when provided with the teachings of the instant application, which includes the incorporated teachings of Kwak, would be fully enabled to make and use the claimed invention, and that the use of the invention does not require undue experimentation.

Applicant maintains that the Examiner has provided **no** evidence that contravenes the knowledge in the art regarding the use of B-cell tumor surface antigens in tumor therapy, or that suggests that use of the multivalent compositions of B-cell surface proteins produced by the methods of the present invention to induce an immune response is unpredictable. The references cited by the Examiner in the Office Action of October 21, 2005 (Raychaudhuri, Wu, and Chatterjee) describe methods of using sequential rounds of immunization to create antibody molecules that act as *mimics* of the initial antigens. These methods are not the subject matter expressly claimed in the instant application[1]. These references are not relevant to the
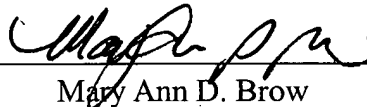
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[1] Note that Applicant does NOT disclaim compositions made using the presently claimed methods that might incidentally function as a mimic of

predictability of the claimed method.

CONCLUSION

For the reasons set forth above, it is respectfully submitted that all reasons for rejection have been addressed and that Applicant's claims should be passed to allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicant encourages the Examiner to call the undersigned collect at (608) 218-6900.

Dated: _____ April 21, 2006 _____

Mary Ann D. Brow
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(..continued)
an antigen.

INDUCTION OF IMMUNE RESPONSES IN PATIENTS WITH B-CELL LYMPHOMA AGAIN THE SURFACE-IMMUNOGLOBULIN IDIOTYPE EXPRESSED BY THEIR TUMORS

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Abstract *Background.* The idiotypic determinants of the surface immunoglobulin of a B-cell lymphoma can serve as a clonal tumor-specific marker, which may have implications for immunotherapy. We sought to determine whether idiotype-specific immune responses against this autologous antigen could be induced in patients with B-cell lymphoma.

Methods. Nine patients were selected who had minimal residual disease or a complete remission after chemotherapy. Each received a series of subcutaneous injections of the immunoglobulin derived from his or her tumor cells (immunoglobulin-idiotype protein), which had been conjugated to a protein carrier and mixed with an immunologic adjuvant.

Results. In seven of the nine patients the injections induced sustained idiotype-specific immunologic responses of the humoral type (two patients), the cell-mediated type (four patients), or both (one patient). The

use of an adjuvant was essential for these immune responses. The induced antibodies bound specifically to autologous immunoglobulin idiotype, inhibited the binding of murine monoclonal antiidiotype antibodies, and bound autologous tumor cells. Cell-mediated responses were demonstrated by the specific proliferation of immune peripheral-blood mononuclear cells to the soluble immunoglobulin-idiotype protein in vitro. The tumors of both of the patients with measurable disease regressed completely. Toxicity associated with the vaccine was minimal and consisted only of mild reactions at the site of intramuscular injection.

Conclusions. These results demonstrate that autologous immunoglobulin idiotype can be formulated into an immunogenic, tumor-specific antigen in humans with B-cell lymphoma, and they provide the background for large-scale trials of active specific immunotherapy of this disease. (N Engl J Med 1992;327:1209-15.)

IMMUNOGLOBULIN molecules are composed of heavy and light chains, which possess highly specific variable regions at their amino termini. The variable regions of heavy and light chains combine to form the unique antigen-recognition site of the immunoglobulin protein. These variable regions contain determinants (molecular shapes) that can themselves be recognized as antigens or idiotypes. B-cell tumors are composed of clonal proliferations of cells synthesizing a single antibody molecule with unique variable regions in the heavy and light chains. B-cell lymphomas are neoplasms of mature resting and reactive lymphocytes, which generally express synthesized immunoglobulin on the cell surface. The idiotypic determinants of the surface immunoglobulin of a B-cell lymphoma can thus serve as a tumor-specific marker for the malignant clone. Studies in animals as well as in humans have demonstrated the usefulness of the immunoglobulin idiotype as a tumor-specific antigen for the study of the biology of B-cell lymphoma in vitro and as a target for passive immunotherapy in vivo.[1-5] Furthermore, active immunization against idiotypic determinants on malignant B cells has produced resistance to tumor growth in several models of syngeneic tumors,[6-16] as well as specific antitumor therapy against established tumors.[17,18] Moreover, preclinical studies in subhuman primates

have demonstrated that optimal immunization with immunoglobulin derived from human lymphomas requires conjugation of the protein to a strongly immunogenic carrier protein (e.g., keyhole-limpet hemocyanin [KLH]) and emulsification in an adjuvant.[19] These results provided the rationale for testing autologous tumor-derived idiotypic surface immunoglobulin as a therapeutic "vaccine" against human B-cell lymphoma.

We immunized nine patients with B-cell lymphoma with the immunoglobulin-idiotype protein derived from their own tumors after they had been treated with chemotherapy and their tumors were in remission. The primary objective of this study was to determine whether immune responses against this autologous antigen could be induced in such patients.

METHODS

Selection of Patients

All patients had a histopathological diagnosis of non-Hodgkin's lymphoma. Immunophenotypic studies of the tumors showed that they were of B-cell origin, with the surface expression of immunoglobulin molecules composed of both heavy and light chains in a monotypic pattern (Table 1). Patients were selected who had minimal residual disease or who were in complete remission after chemotherapy. Seven patients had had only a single regimen of chemotherapy, and two had had multiple regimens (Table 1). The base-line studies used to evaluate clinical disease in all patients before immunization with idiotypic immunoglobulin included complete physical examination, chest radiography, routine blood counts and chemistry tests, and bipedal lymphangiography, with or without abdominal and pelvic CT scanning. None of the patients had received any chemotherapy for at least six months, and none received antitumor therapy during the study. All underwent restaging during immunization and at the completion of the series of immunizations, according to the objective procedures used at base line. In addition, three patients with rapidly progressive recurrent lymphoma were enrolled in a separate study to assess safety; all three

required reinstitution of chemotherapy shortly after enrollment, did not complete the series of immunizations, and were not studied further.

Isolation and Conjugation of Autologous Immunoglobulin Idiotype

Lymphoma cells from a biopsy specimen were fused with the hypoxanthine–aminopterin–thymidine (HAT)–sensitive heterohybridoma K6H6/B5.[20] Hybrid cells selected from HAT medium that secreted immunoglobulin with the type of heavy and light chains corresponding to the known immunophenotype of the tumor specimen were expanded into larger volumes (1 liter), and the supernatants were collected and pooled.

The tumor immunoglobulin-idiotype protein was purified from bulk supernatant by affinity chromatography (antihuman immunoglobulin–Sepharose). After the protein was adsorbed to the immunoadsorbent matrix, the immunoglobulin idiotype was eluted with 0.1 M glycine–hydrochloric acid buffer (pH 2.4). The purity of the protein (>90 percent) was determined by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis. Before conjugation, hemocyanin from the keyhole limpet (*Megathura crenulata*) (Calbiochem, San Diego, Calif.) was passed over a QAE Zeta Prep 15 disk (LKB, Broma, Sweden) to reduce the level of endotoxin to less than 1000 EU (endotoxin units) per milliliter. Equivalent volumes of immunoglobulin idiotype and KLH (both at a concentration of 1 mg per milliliter and sterilized by filter) were mixed together, and sterile glutaraldehyde (Sigma, St. Louis) was added, for a final concentration of 0.1 percent. The immunoglobulin–KLH conjugate was then dialyzed extensively against physiologic saline. The final product was tested for sterility and general safety before it was used.

Immunologic Adjuvant

The immunologic adjuvant used in this study was the vehicle component of SAF-1 (Syntex adjuvant formulation 1) described by Allison and Byars.[21] It is composed of 5 percent (wt/vol) squalane (BASF, Parsippany, N.J.), 2.5 percent Pluronic L121 polymer (Aldrich Chemical, Milwaukee), and 0.2 percent polysorbate (Tween 80, Sigma) in phosphate-buffered saline.

Assays for Serum Antiidiotypic Antibody

Direct Enzyme-Linked Immunosorbent Assay

For the direct enzyme-linked immunosorbent assay (ELISA), preimmune and hyperimmune serum samples from each patient were diluted over wells of a microtiter plate that had been coated with either autologous immunoglobulin idiotype or a panel of four isotype-matched human tumor immunoglobulins of unrelated idiotype. Bound antibody was detected with horseradish peroxidase–goat antihuman light-chain antibodies directed against the light chain not present in the immunoglobulin idiotype (Caltag Laboratories, South San Francisco).

Inhibition ELISA

Preimmune and hyperimmune serum samples from each patient were incubated on microtiter plates that had been coated with autologous immunoglobulin idiotype. Each plate was washed, and then a murine monoclonal antiidiotype antibody was added to the wells. Bound murine monoclonal antibody was detected with horseradish peroxidase–conjugated goat antimouse IgG antibodies (Caltag).

Inhibition Cell-Binding Assay

Tumor cells were obtained from a cryopreserved lymph-node-biopsy specimen obtained before treatment; the specimen was shown by flow cytometry to have 21 percent CD3-positive T cells (antihuman Leu-4 fluorescein isothiocyanate, Becton Dickinson, San Jose, Calif.), 4 percent lambda-light-chain–bearing normal B cells (fluorescein isothiocyanate–goat F(ab')₂ antihuman lambda, Tago, Burlingame, Calif.), and 75 percent kappa-light-chain–

Table 1. Characteristics of Patients with B-Cell Lymphoma.

Patient No. (Sex/Age)	Histologic Subtype*	Surface Immuno-globulin	Prior Therapy† (No. of Cycles)	Months since Last Therapy	Site of Measurable Disease
Group 1					
1 (F/41)	FM	IgM lambda	CVP (8)	9	Submandibular lymph node
2 (M/56)	FM	IgA lambda	CVP (10)	8	None
3 (M/47)	FSC	IgM kappa	Chl/P (12)	6	None
4 (F/43)	FSC→DM	IgM kappa	mAb, CEPP (4), ABMT	7	Cutaneous mass
5 (M/59)	FSC	IgM kappa	CVP (9)	8	None
Group 2					
6 (M/45)	FSC	IgM kappa	CVP (10)	6	None
7 (M/50)	FM	IgM kappa	CVP (8)	18	None
8 (F/25)	FSC	IgM kappa	CVP (9)	20	None
9 (M/56)	FLC	IgG kappa	[131I]anti-CD20, ABMT	12	None

*FM denotes follicular mixed large cell and small cleaved cell; FSC follicular small cleaved cell; FSC→DM follicular small cleaved cell, with transformation to diffuse mixed large cell and small cleaved cell; and FLC follicular large cell.

†CVP denotes cyclophosphamide, vincristine, and prednisone; Chl/P pulsed chlorambucil and prednisone; mAb antiidiotype monoclonal antibody; CEPP cyclophosphamide, etoposide, prednisone, and procarbazine; ABMT autologous bone marrow transplantation; and [131I]anti-CD20 radiolabeled monoclonal antibody.

bearing tumor cells (fluorescein isothiocyanate–goat F(ab')₂ antihuman kappa, Tago) (data not shown). Indirect staining with a murine antiidiotype monoclonal antibody revealed binding of 61 percent of the lymph-node cells.

Cryopreserved cells (1×10⁶) prepared by Ficoll–Hypaque centrifugation were incubated first with 50 μl of hyperimmune or preimmune serum or 1 μg of unlabeled antiidiotype monoclonal antibody in a total volume of 100 μl at 4°C for 30 minutes. After washing, the cells were incubated with a saturating concentration of biotinylated antiidiotype monoclonal antibody at 4°C for 30 minutes, washed twice again, and then incubated with streptavidin–phycoerythrin (Becton Dickinson) at 4°C for 30 minutes. They were then washed twice, fixed in 2 percent paraformaldehyde, and analyzed with a cell sorter (FACS 440, Becton Dickinson).

Purification of Human Antiidiotypic Antibody from Hyperimmune Serum

Twenty milliliters of hyperimmune serum was passed over an immunoadsorbent column consisting of autologous immunoglobulin idiotype coupled to Sepharose 4B, and antiidiotypic antibodies were eluted with 0.1 M glycine–hydrochloric acid buffer (pH 2.4). The presence or absence of antibodies of the IgG class in the purified preparation of antiidiotypic antibodies was determined with a detector consisting of horseradish peroxidase–goat antihuman IgG antibodies (Tago) specific for the gamma heavy chain. The presence of mu and gamma heavy chains and both kappa and lambda light chains was established by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis.

Assay for Idiotype-Specific Proliferative Response

Proliferation assays were adapted from the techniques of Chain et al.[22] Fresh peripheral-blood mononuclear cells (PBMCs), prepared by Ficoll–Hypaque gradient centrifugation, were washed and plated at a concentration of 4×10⁵ cells per well in Iscove's modified Dulbecco's medium (IMDM) with 1 percent human AB serum (IMDM-1 percent AB). KLH or autologous immunoglobulin idiotype at concentrations of 0 to 100 μg per milliliter in IMDM-1 percent AB preparation was added in quadruplicate. After the cells were incubated for three days at 37°C in an atmosphere containing 5 percent carbon dioxide, they were transferred to a preparation of IMDM and 5 percent fetal-calf serum containing recombinant interleukin-2 (30 U per milliliter, a gift from Cetus, Emeryville, Calif.). The plate was then incubated for two days and pulsed for 16

	SITE OF
SINcE LAST THERAPY	MEASURABLE DISEASE
9	Submandibular lymph node
8	None
6	None
7	Cutaneous mass
IT	
8	None
6	None
18	None
20	None
12	None

I cell; FSC follicular small cleaved mation to diffuse mixed large cell

isone; Chl/P pulsed chlorambucil :PP cyclophosphamide, etoposide, row transplantation; and [131I]anti-

inate–goat F(ab')₂ anti-
Indirect staining with a
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by Ficoll–Hypaque cen-
µl of hyperimmune or
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ive Response

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cells (PBMCs), prepared
in, were washed and plat-
well in Iscove's modified
ercent human AB serum
ous immunoglobulin idio-
per milliliter in IMDM-
quadruplicate. After the
°C in an atmosphere con-
re transferred to a prepa-
erum containing recombi-
ift '   Cetus, Emeryville,
iwc   and pulsed for 16

to 20 hours with ³H-labeled thymidine (1 µCi per well). Data are expressed as mean (±SEM) counts per minute of [³H]thymidine incorporation.

Expansion of Immune PBMCs

Initial five-day cultures of PBMCs established as described above were expanded in the IMDM–5 percent fetal-calf serum containing interleukin-2 (30 U per milliliter) for three weeks. Harvested cells were replated in IMDM–1 percent AB containing autologous immunoglobulin idiotype and fresh irradiated (5000 R) autologous PBMCs (4×10^5 cells per well) as antigen-presenting cells for five days, before pulsing with [³H]thymidine.

RESULTS

Immunization Schedule

All patients received subcutaneous injections of 0.5 mg of immunoglobulin-idiotype protein conjugated to KLH at time 0 and at 2, 6, 10, and 14 weeks, followed by booster injections at 24 and 28 weeks. Patients in the first trial (group 1) received immunoglobulin idiotype–KLH alone for the first three immunizations, then immunoglobulin idiotype–KLH emulsified in the Pluronic polymer-based adjuvant formulation for all subsequent immunizations. Although marked responses to KLH were observed after a single immunization, no idiotype-specific immune responses were evident before the adjuvant was added to the program for this first group of patients. Therefore, patients in the second trial (group 2) received the entire series of immunizations with this adjuvant. In all nine patients, the production of 'idiotype-specific antibody and the proliferative responses of PBMCs in vitro were determined immediately before each immunization and one to two months after the last immunization. The KLH carrier served as a convenient internal control for the immunocompetence of each patient. All patients had both humoral-antibody and PBMC proliferation to the KLH protein except Patient 4, who had only the latter response.

Humoral Responses to Idiotype

Antibody responses were screened by analysis of preimmune and immune serum samples collected at various times in the direct ELISA (see the Methods section). Responses indicated by a significant increase in the level of antibody binding to autologous immunoglobulin idiotype in hyperimmune serum as compared with preimmune serum were then confirmed by a competition ELISA (see the Methods section). Three patients were classified as having an antibody response on the basis of this two-step analysis (Fig. 1 and 2, showing both types of assays). It is clear that immunization with autologous immunoglobulin-idiotype protein induced titers of antiidiotypic antibody that either directly bound (Fig. 1) or inhibited the binding (Fig. 2) of a murine antiidiotype monoclonal antibody to immunoglobulin idiotype on the plate. The specificity of the humoral response for the idiotype of the immunoglobulin was demonstrated by a lack of binding of hyperimmune se-
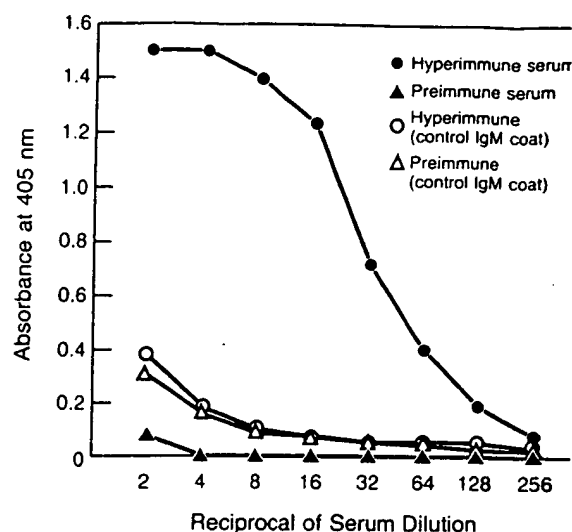

Figure 1. Serum Antiidiotypic-Antibody Titers Induced after Repeated Immunizations with Autologous Immunoglobulin Idiotype in Patient 1.

Preimmune and hyperimmune serum samples from the patient were diluted over wells of a microtiter plate that had been coated with either autologous immunoglobulin idiotype or a panel of four isotype-matched human immunoglobulins of unrelated idiotype. Bound antibody was detected with horseradish peroxidase–conjugated goat antihuman light-chain antibodies directed against the light chain not present in the immunoglobulin idiotype. For clarity, only one representative immunoglobulin coat of irrelevant idiotype is shown, in which there is a lack of marked binding activity of these serum samples.

rum to a panel of isotype-matched human immunoglobulins of unrelated idiotype (Fig. 1) or by lack of inhibition of a panel of heterologous idiotype–antiidiotype systems (Fig. 2). Furthermore, the inhibition of each antiidiotype-monoclonal-antibody reagent was specific; that is, each monoclonal antibody could be blocked from binding to its corresponding immunoglobulin idiotype only by hyperimmune serum obtained from the same patient (data not shown).

The kinetics of the antibody responses elicited are shown in the screening ELISAs represented in Figure 3. Increases in the serum titer of antibody binding autologous immunoglobulin idiotype were not detectable in Patient 1 (group 1) until after the fourth immunization in the series (Fig. 3). In contrast, both responding patients from group 2 had increases in antibody titers at earlier points, with increases first observed after a single immunization (Fig. 3; Patient is representative of both these patients). In each patient, these increases in antibody titer peaked after the fifth immunization. The in vitro responses of these three patients have persisted at peak levels for at least 9 months (range, 9 to 15).

The antiidiotypic antibodies produced in Patient were purified by affinity chromatography and were shown to contain heterogeneous light chains as well a

Patient 7

Patient 8

Reciprocal of Serum Dilution

● Hyperimmune serum
▲ Preimmune serum
○ Hyperimmune (control IgM coat)
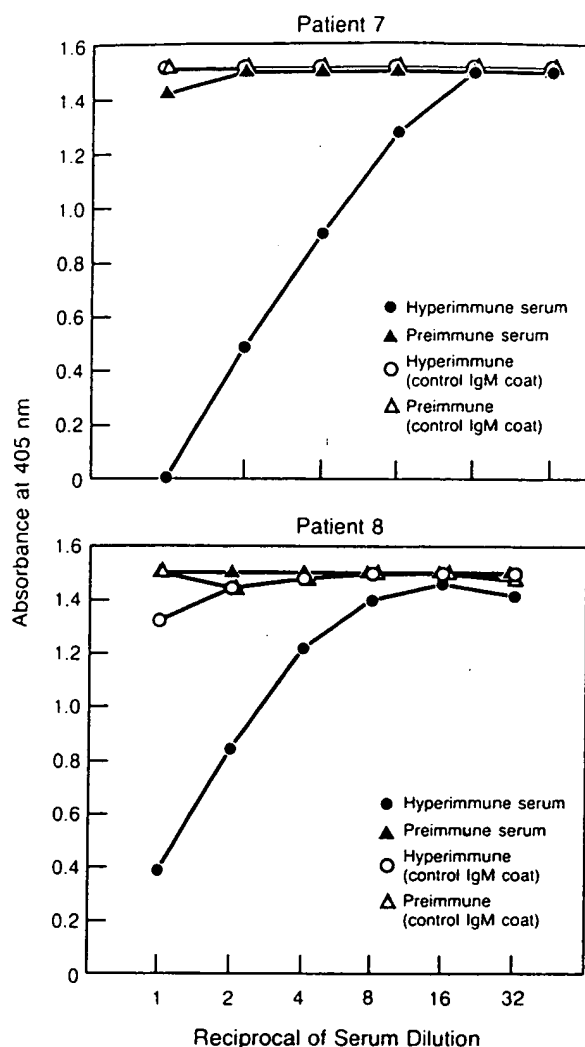△ Preimmune (control IgM coat)

Figure 2. Inhibition of Binding of a Murine Antiidiotype Monoclonal Antibody to Autologous Immunoglobulin Idiotype by Hyperimmune Serum in Patients 7 and 8.

Preimmune and hyperimmune serum samples were diluted over microtiter plates that had been coated with autologous immunoglobulin idiotype. The plates were washed, and culture supernatant containing a corresponding murine antiidiotype monoclonal antibody was added to the wells. Bound murine monoclonal antibody was detected with horseradish peroxidase–conjugated goat antimouse IgG antibodies. The specificity of the hyperimmune samples was tested by coating plates with a panel of isotype-matched immunoglobulins of unrelated idiotype and substituting for the antiidiotype monoclonal antibody an antiidiotype monoclonal antibody corresponding to the respective control IgM coat. Each panel shows a lack of inhibition of one representative heterologous immunoglobulin idiotype–antiidiotype system by each of the serum samples.

IgG heavy chains. The presence of antibodies of the IgG class was confirmed by a specific ELISA (see the Methods section). This patient's antibody titer was successfully boosted with a single administration of immunoglobulin idiotype–KLH in adjuvant when the humoral response declined after a period of 15 months (data not shown).

Antibody Binding to Autologous Tumor

We also tested the ability of the idiotype-specific humoral response to bind autologous tumor cells in vitro. As shown in Figure 4, the binding of a labeled murine antiidiotype monoclonal antibody to tumor cells from a lymph-node specimen obtained from Patient 8 before treatment was inhibited by her hyperimmune serum but not by her preimmune serum. The specificity of the blocking by the hyperimmune serum from this patient was confirmed by the lack of inhibition of a heterologous antiidiotype monoclonal antibody binding to tumor cells from another patient expressing an isotype-matched immunoglobulin of

unrelated idiotype under the same experimental conditions (data not shown). Affinity-purified antiidiotypic antibodies from the hyperimmune serum of the two other patients who had idiotype-specific humoral responses were also demonstrated to bind autologous tumor cells (data not shown).

Cellular Responses to Idiotype

Cellular immune responses were measured by the proliferation of PBMCs in response to KLH and to autologous immunoglobulin idiotype at concentrations ranging from 1 to 100 μg of soluble protein per milliliter in five-day in vitro cultures. Table 2 shows the results of assays of peak hyperimmune PBMCs from all patients. None of the preimmune PBMCs demonstrated any preexisting proliferation in response to autologous immunoglobulin idiotype that was greater than the proliferation in culture medium alone (data not shown). Hyperimmune PBMCs from all patients showed strong proliferative responses to the KLH carrier. The finding of primary interest was the hyperimmune proliferative responses to immunoglobulin idiotype that were detected in five patients. Although their responses were of a lower magnitude than parallel responses to KLH, Patients 3, 4, 6, 8, and 9 were classified as responders on the basis of reproducible increases, sustained over multiple time points, in the levels of activity in wells containing immunoglobulin idiotype, as compared with levels in wells containing medium alone. Patients who had occasional increases in activity in wells containing immunoglobulin idiotype as compared with medium alone were classified as nonresponders (Patients 1 and 5).

Increases in proliferation in response to autologous immunoglobulin idiotype in the two responding patients in group 1 were not detectable until after the fourth immunization of the series. The proliferative responses in the three responding patients in group 2 required a minimum of two immunizations. However, in two of these patients, peak responses were observed after additional immunizations had been administered (Table 2, Patients 8 and 9). These proliferative re-
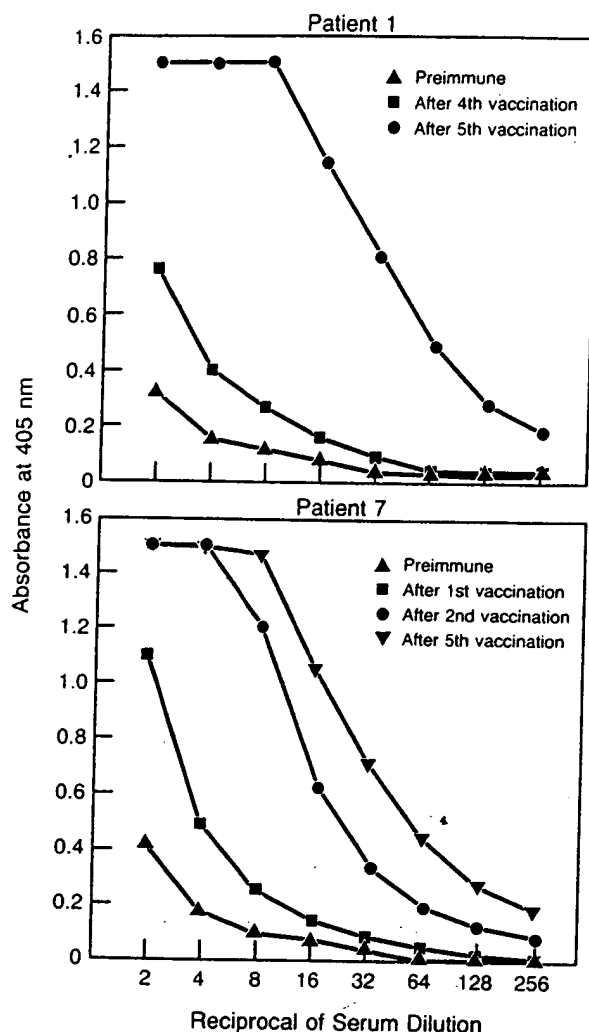
Patient 1

Patient 7

Figure 3. Kinetics of the Humoral Antiidiotypic Responses Elicited by Immunization with Immunoglobulin Idiotype in Patients 1 (Group 1) and 7 (Group 2).

The serial serum antibody titers in each panel were measured in a single screening ELISA as described in the legend to Figure 1.

sponses to immunoglobulin idiotype in both groups of patients have persisted during 9 to 14 months of follow-up.

Flow-cytometric analysis of cultures demonstrating proliferation in response to immunoglobulin idiotype revealed a predominance of cells staining positively for CD4 (>95 percent), suggesting the phenotype of the responding cell subpopulation (data not shown). These cultures could be successfully expanded for approximately four weeks by stimulation alternately with interleukin-2 and immunoglobulin idiotype–pulsed autologous irradiated PBMCs as antigen-presenting cells (Fig. 5). The specificity of the responses for immunoglobulin idiotype was confirmed by a lack of proliferation in response to an isotype-matched human immunoglobulin

of unrelated idiotype, as compared with medium alone (data not shown).

Toxicity and Disease Activity

Toxicity was minimal in all 12 patients (including the 3 with rapidly progressive disease). All patients had transient local reactions characterized by mild erythema, induration, and discomfort, without skin breakdown at the injection sites. Administering the components of the vaccine (immunoglobulin idiotype–KLH and adjuvant) separately in one patient with a moderate local reaction and in another with a moderate systemic reaction characterized by fever, rigors, and diffuse arthralgias revealed that the adjuvant was the component associated with these reactions. Both of these moderate reactions resolved completely after 24 to 48 hours. The only laboratory abnormality associated with immunization was a mild elevation (less than twice the normal value) in the serum creatine kinase concentration 24 hours after immunization in an occasional patient.

All patients were also closely monitored for disease activity by means of repeated physical examinations and routine laboratory and radiographic studies. Of the two patients with measurable tumor at the initiation of immunization, one (Patient 1) had complete regression of a single 2.5-cm left submandibular lymph node, documented by CT, and the other (Patient 4) had complete regression of a 4.5-cm cutaneous
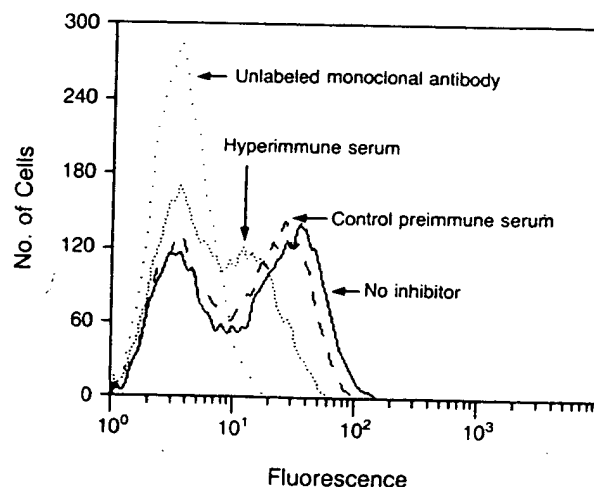

Figure 4. Inhibition of Binding of a Murine Antiidiotype Monoclonal Antibody to Tumor Cells from Patient 8 by Her Hyperimmune Serum.

Single-color fluorescence histograms from a single experiment represent staining of lymphoma cells with a biotinylated antiidiotype monoclonal antibody after incubation of the cells with hyperimmune serum containing antiidiotypic antibody, control preimmune serum, or unlabeled monoclonal antibody or without inhibitor. The specificity of the hyperimmune serum from this patient was confirmed by the lack of inhibition of a heterologous antiidiotype-monoclonal-antibody reagent binding to tumor cells from another patient expressing immunoglobulin of the same isotype under the same experimental conditions (data not shown).

Table 2. In Vitro Proliferative Responses of PBMCs to KLH and Immunoglobulin Idiotype.

Patient No	No. of Immunizations	Proliferation in Medium	Response to KLH			Response to Immunoglobulin Idiotype		
			10 µg/ml	50 µg/ml	100 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml
			mean (±SEM) counts per minute for [³H]thymidine incorporation by PBMCs*					
Group 1								
1	5	1,721±612	5,459±1305	6,938±402	ND	747±234	3,117±689	ND
2	6	137±55	2,153±560	2,697±731	ND	188±33	355±40	ND
3	5	14,003±1827	34,799±4906	58,190±1816	83,646±3768	14,394±6177	22,764±5264	40,102±4036
4	4	4,477±584	6,767±531	16,020±1517	10,541±560	9,629±596	7,380±382	8,182±1138
5	7	4,380±394	24,577±898	ND	31,058±781	5,362±463	7,366±372	ND
Group 2								
6	2	12,887±1435	39,996±2826	91,237±1965	113,508±5139	32,922±3282	29,584±5167	ND
7	5	6,702±544	54,957±2753	ND	67,571±7341	7,162±1179	ND	5,779±481
8	5	7,645±731	71,152±3172	ND	81,842±3162	10,769±341	ND	17,977±1334
9	6	8,953±767	68,118±3858	ND	67,657±1013	6,419±997	ND	20,332±1379

*Each mean (quadruplicate wells) was calculated from assays of peak hyperimmune autologous PBMCs. ND denotes not done.

lymphomatous mass on the right arm. These patients remain in remission 24 and 10 months, respectively, after completing immunization. Of the patients who were in remission and completed the series of immunizations, one has had a recurrence (Patient 5); all the other patients remain in remission after a median follow-up of 10 months.

DISCUSSION

The idiotype of the immunoglobulin expressed on the surface of a B-cell lymphoma cell can function as a tumor-specific antigen. A major obstacle limiting the success of passive immunotherapy of B-cell lymphomas with a murine antiidiotype monoclonal antibody has been the emergence of tumor cells with mutated forms of their surface immunoglobulin.[23] Active immunization with purified immunoglobulin, by virtue of induction of a polyclonal immune response directed against multiple idiotypic determinants on this molecule, may overcome this obstacle. The results presented here show that immunoglobulin idiotype can serve as an immunogen (antigen) eliciting both humoral and cell-mediated immunologic responses. Seven of nine patients with B-cell lymphoma had sustained idiotype-specific immune responses of a humoral type (two patients), cell-mediated type (four patients), or both types (one patient). The responses specific for this autologous protein were significantly increased over base-line levels, although they were not equal in magnitude to those generated against the KLH carrier. This was an expected result, given the strongly immunogenic nature of the exogenous antigen as compared with an autologous protein. The humoral antiidiotypic response elicited was clearly capable of binding autologous tumor in vitro. We presume that this induced antibody bound to tumor cells in vivo; however, this could not be proved because at the time of their immune responses the patients
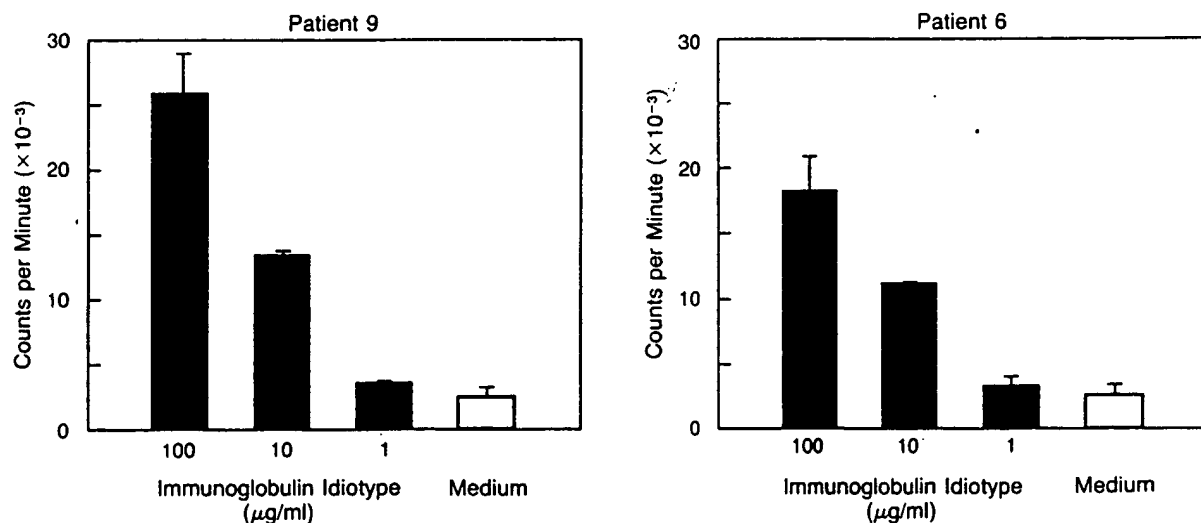

Figure 5. Expansion and Restimulation of Immune PBMCs in Patients 9 and 6.
PBMCs proliferated in vitro in response to autologous immunoglobulin idiotype after expansion of the primary five-day cultures (described in the Methods section) with interleukin-2 for three weeks and restimulation with idiotypic-immunoglobulin protein at various concentrations. Values are means ±SEM.

were all in complete clinical remission. The present study also establishes the requirement for an immunologic adjuvant, such as the Pluronic polymer-based vehicle used.

The concept of a therapeutic tumor vaccine might involve two different strategies. The goal of one approach is to induce immune resistance in the host against the regrowth of tumor cells that remain after primary therapy. Such an approach is exemplified by clinical trials of whole-tumor-cell or cell-lysate vaccines administered to patients with colon cancer[24] and to patients with melanoma[25] who are at high risk for relapse after standard therapy. The alternative approach is to administer the vaccine as therapy for clinically apparent disease. Several trials in which melanoma-cell-lysate vaccines have been administered without prior cytoreductive therapy have reported objective antitumor responses. In these studies, the induction of tumor-specific immunologic responses appears to be a requisite for the clinical responses observed, and the inability to elicit immune responses in the presence of gross tumor is a factor limiting the success of this approach.[26-28]

In this study we adapted a vaccine approach to B-cell lymphomas. We reasoned that the group in which we would have the best chance to demonstrate that patients could mount an immune response to components of their own tumor would be patients whose tumors were in remission but whose previous therapy had been limited. It was possible to induce a low level of specific humoral and cellular immunity in such patients. There may be considerable latitude for optimizing the immunogenicity of the vaccine by modifying its "foreignness" by molecular engineering and by using better immunologic adjuvants. If more-potent vaccines can be formulated, then the approach can be extended to immunization of patients with greater tumor burdens. The successful induction of immune responses to the KLH carrier suggests that chemotherapy-induced immunosuppression is not an obstacle to active immunotherapy administered as an adjunct to cytoreductive drug therapy.

A potential limitation of this approach is that the use of autologous immunoglobulin-idiotype protein as the immunogen requires that a vaccine be produced individually for each patient and that accessible tumor tissue be available as starting material. Methods to refine and streamline the production of the purified immunoglobulin-idiotype product would thus be desirable.

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